

## Season, sex and flight muscle investment affect take-off performance in the hibernating small tortoiseshell butterfly *Aglais urticae* (Lepidoptera: Nymphalidae)

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**Abstract.** Flight ability is generally expected to increase with relative flight muscle mass. Changes in weight can therefore be expected to influence the capacity to rapidly take-off, which can determine mating success and predator avoidance. This study examined the influence of relative flight muscle mass, sex, and season on free take-off flight ability in a butterfly model (*Aglais urticae*) that undergoes adult winter hibernation. Mass change and take-off flight ability (velocity and take-off angle), was predicted to fluctuate with season (before, during and after hibernation) and sex (due to reproductive investment). Our results indeed showed changes in take-off ability in relation to both parameters. Females maintained velocity across seasons but reduced take-off angles during and after hibernation. Male flight speed increased during and after hibernation, whereas take-off angles were significantly reduced during hibernation. Finally, we showed that investment in relative flight muscle mass increased velocity in female, but not in male butterflies.

**Key words:** Flight, diapause, Lepidoptera, predation, body mass, thorax mass.

### INTRODUCTION

In many species the development of a highly agile body, which may be beneficial as an anti-predation strategy, comes at the cost of reproduction, or vice versa (*e.g.* lizards: Shine, 1980; fish: Plaut, 2002; Ghalambor *et al.*, 2004; Evans *et al.*, 2007; prawns: Berglund & Rosenqvist, 1986; birds: Swaddle & Witter, 1997; scorpions: Shaffer & Formanowicz, 1996). In winged insects such as butterflies, flight is one of the primary ways of evading an attacking predator. Flight speed has been shown to reduce bird capture rates of butterflies (Chai & Srygley, 1990) suggesting that it is indeed a major determinant of escape

ability. Climbing flight (net upward movement), as is common in prey birds (Hedenström & Rosén, 2001), is potentially another way of outmanoeuvring predators. Because winged animals with smaller body masses are aerodynamically favoured during ascent, prey animals may increase their chances of survival by choosing a steeper take-off as their generally larger predators are often unable to match the climbing rate (Hedenström & Rosén, 2001). As butterflies are often targeted by avian predators while feeding or resting (Morse, 1975) managing the transition from perching to airborne may be particularly important. Perching male butterflies also require the ability to quickly depart in order to intercept passing females (Wickman, 1992; Van Dyck, 2003). A high proportion of flight muscle mass relative to body mass is generally expected to enable more precise and rapid flight (Srygley & Dudley, 1993; Almbro & Kullberg, 2008). Insects are, however, highly sensitive to the costs associated with flight (Roff, 1984) suggesting that weight increases (thus reducing relative flight muscle mass) has the potential to markedly influence flight speed or trajectory (Srygley & Dudley, 1993). Our understanding of take-off flight behaviour in a predation context, however, is limited in insects that carry substantial and naturally fluctuating body weight, and has rarely been tested in free flight (but

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see Srygley & Kingsolver, 1998; Almbro & Kullberg, 2008, 2009).

In butterflies, adult winter hibernation demands large amounts of energy (i.e. abdominal fat) to be accumulated prior to hibernation in order to ensure survival until activity is resumed. A lipid content of as much as 25% of body mass is not unusual (Pullin, 1987). Whereas a substantial weight increase prior to hibernation constrains butterfly escape flight speed (Almbro & Kullberg, 2008), no information has been gathered concerning flight ability after hibernation when the demand for flight capacity remains high. Not only should lipid loads be reduced during hibernation (thus increasing relative flight muscle mass), mating would also be expected to result in sex-specific changes in body mass in species in which reproduction occurs after hibernation. Whereas both sexes need to accumulate and deplete lipid loads in relation to hibernation, we would expect females to carry relatively more weight than males after hibernation as substantial gamete related loads are transferred from male to female upon mating (Svård & Wiklund, 1989). Males on the other hand are likely to increase their relative flight muscle mass as weight is lost following hibernation and mating. However, while the period spent in hibernation may deplete the lipid storage and increase relative flight muscle mass, disuse can result in degeneration of flight muscles (Stegwee *et al.*, 1963) that may hinder the ability to escape predators or locate mates (Layne & Rice, 2003). Adult butterflies occasionally emerge from hibernation for short periods during favourable weather conditions, yet their flight ability at such times has to our knowledge not been tested previously.

In this study we investigated differences in take-off flight performance in relation to sex and season after a simulated predator attack in wild caught small tortoiseshell butterflies (*Aglais urticae*, Linnaeus 1758). Using a 3D-tracking camera system, male and female free take-off flight ability was tested i) before hibernation ii) during hibernation iii) after hibernation. We predicted that flight ability (velocity and take-off angle) would be higher in individuals with a relatively larger flight muscle mass, and that changes in relative flight muscle mass would be related to sex and season. We thus expected male butterflies to increase flight ability with reduced body mass due to lipid depletion during hibernation and mating after hibernation; with the exception for lipid depletion during hibernation, female butterflies were expected to exhibit little or no loss in body mass after hibernation due to reproduction, and thus we did not expect an increase in female flight ability after hibernation.

## MATERIALS AND METHOD

In Sweden, adult small tortoiseshells hibernate from autumn to spring in dark sheltered areas and emerge in early spring to mate. The butterflies used in this study were collected in winter (January-February) and spring (March-April), 2006 and 2007 (data was pooled as t-tests of morphological measurements revealed no significant differences between years) nearby Tovetorp Zoological Research Station, located in South-East Sweden (58°56'N 17°08'E). Two groups of male and female butterflies were used: i) hibernating butterflies that were collected late in the winter (end of January/early February) whilst sitting in dark, unheated attics and barns near the research station (referred to henceforth as butterflies "during hibernation"), and ii) actively flying individuals captured with a net around the research station in early spring (end of March/early April; referred to henceforth as butterflies "after hibernation").

Butterflies collected during hibernation were kept in a dark incubator (Termaks KB8000, Bergen, Germany) set to  $4 \pm 0.1^\circ\text{C}$  until treatments commenced around the same time as small tortoiseshells were observed to emerge, and were captured in the wild. The temperature in the incubator was at that point raised to  $8 \pm 0.1^\circ\text{C}$  and both hibernating and active butterflies were kept in the incubator until used in flight trials. The incubator temperatures approximate outdoor averages for the region during the specified months. Prior to flight trials all butterflies were kept in indoor cages (0.65 x 0.65 x 0.70 m) and allowed to fly for 4 hours. The cages were furnished with moist paper towels to prevent dehydration. In addition to natural light in the room shining through windows, extra light was provided by two Philips Powertone HPI-T Plus 400W light bulbs. Before being subjected to trials, the butterflies spent a minimum of 30 minutes and a maximum of 3 hours in the incubator to facilitate handling. Because all butterflies were transferred back to the incubator at the same time after the allotted 4 hours in the flight cages, some spent more time in the incubator as flight trials were carried out in a randomised order. However, because the butterflies remain inactive whilst incubated, we do not believe that small variations in incubation time in any way affected the flight performance. To further contrast seasonal effects in flight ability, the data from the current study were compared to flight data collected in a previous study on small tortoiseshell butterflies tested prior to hibernation (referred to in the current paper as butterflies 'before hibernation', Almbro & Kullberg, 2008). All methods in the previous study were the same as in the current

study, except that half of the butterflies tested before hibernation had access to sugar water, whereas none of the butterflies tested during/after hibernation were supplied with food. However, food accessibility did not result in differences in body measurements in butterflies before hibernation (Student's t-test;  $n_{\text{unfed}}=14$ ;  $n_{\text{fed}}=11$ : body mass:  $t=0.6$ ,  $p=0.5$ ; abdomen mass:  $t=1.3$ ,  $p=0.2$ ; FMR:  $t=-0.9$ ,  $p=0.4$ ), nor did fed and unfed butterflies differ in velocity (GLM:  $F=0.5$ ,  $p=0.5$ ) or take-off angle (GLM:  $F=1.9$ ,  $p=0.2$ ) when controlling for sex, relative flight muscle mass and type of flight (escape/control).

### Flight trials

All flight trials were conducted in an indoor experimental area (3 x 4.7 x 2 m) illuminated by eight high frequency natural light fluorescent tubes (Philips TL5 HO 54W) in the ceiling and a spotlight (Philips Broadway MSR 200; high-efficiency hot restrike metal halide lamp with UV-light). Room temperature was maintained at  $20 \pm 1^\circ\text{C}$ . Biobserve Track-it 3D-camera system (Gmbtl, Bonn, Germany) was used for recording all butterfly flights, providing 50 x-, y-, and z-coordinates per second. Every butterfly was tested once and was either attacked by a model predator or allowed to take off spontaneously. The model predator consisted of a black cardboard box (0.2 m X 0.15 m X 0.15 m) attached to a cart on a rail released 2 m from the butterfly perch at a 14 degree incline (detailed description in Almbro & Kullberg, 2008). All butterflies were allowed to warm up for a minimum time of 3 minutes and a maximum of 5 minutes. After each trial, butterflies were cooled for about 15 minutes, weighed to the nearest mg (Precisa 205A SCS, Dietikon, Switzerland) to obtain body mass (total wet weight), killed by freezing ( $-18^\circ\text{C}$ ), and dissected to obtain thorax and abdominal weights and to determine the sex. Because it consists largely of flight muscles, the weight of the thorax (after wings and legs are removed) is generally considered a reliable proxy for flight muscle mass. Lipids and gametes are located in the abdomen, allowing reliable estimates of lipid accumulation and reproductive load.

### Statistical analyses

Butterfly flight data was analysed by using the coordinates provided by the Track-it 3D camera system and via Track 3d (computer software made for analysing space-time data by Ulf Norberg, Stockholm University) calculating velocity and take-off angle for every individual flight at 0.1; 0.2, 0.3; 0.5; 0.7 m from

the start. Flight velocity (m/s) was calculated when butterflies passed each of the five distances from the start by measuring the distance between two successive coordinates and dividing by the time between the two recorded coordinates. Take-off angle was calculated for each of the five distances as the angle between horizontal and a line drawn between the perch and the height of the butterfly at that distance. Statistical tests used were ANOVA for morphological comparisons, General linear model (GLM), with the five flight distances as repeated measure, for analysing the effect of sex, season and morphology on flight measurements, and linear regression for analysing correlations between morphological measurements and flight parameters. Body mass was always included as a covariate with thorax mass in the GLMs to analyse relative flight muscle investment and its effect on the measured flight parameters. All data were normally distributed and equality of variances was established with Levene's test. All statistical analyses were made using Statistica version 8.0 (StatSoft, Inc. 1984-2008, Uppsala, Sweden).

## RESULTS

A total of 92 hibernated butterflies were used in this study, of which 28 males and 22 females were collected whilst hibernating, and 30 males and 12 females were captured whilst flying actively in the wild after hibernation. To compare flight ability between seasons data from 24 males and 28 females were used from an earlier study on the flight ability of small tortoiseshells prior to hibernation (Almbro & Kullberg, 2008).

Most butterflies took off in a relatively straight manner and headed towards the ceiling or the wall facing them. Only a handful would fly around the room and few flights lasted longer than 5 seconds and most left the tracking area after approximately 1 m. Butterflies that were attacked by the model predator flew faster than control butterflies (GLM with velocity as repeated measurement, and sex, season and type of flight (escape/control) as categorical factors; effect of type of flight:  $F_{1,113}=6.03$ ,  $p=0.02$ ). Therefore, further statistical analyses were only conducted on attacked butterflies (Females:  $N_{\text{before hibernation}}=17$ ,  $N_{\text{during hibernation}}=13$ ,  $N_{\text{after hibernation}}=10$ ; Males:  $N_{\text{before hibernation}}=8$ ,  $N_{\text{during hibernation}}=16$ ,  $N_{\text{after hibernation}}=9$ ).

### Morphological comparisons between sex and season

Male butterflies showed a pattern of reduced body mass after hibernation (Table 1, Fig. 1A), with abdomen mass being highest prior to hibernation and lowest after

hibernation, whereas thorax mass did not change across seasons (Table 1, Fig. 1B-C). Female butterflies showed no change in any of the morphological measurements across seasons (Table 1, Fig. 1 A-C). Male and females butterflies did not differ in body mass before or during hibernation, but males were lighter after hibernation. Thorax mass did not differ between the sexes at any time (Table 1, Fig. 1A-C).

#### Effect of sex, season and morphology on flight ability

Take-off angle tended to differ between the sexes in butterflies tested after hibernation (Table 2, Fig. 2). Males and females did not differ in velocity across seasons; however, there was an interaction between sex and distance from start which revealed females to fly faster than males, but only later in the flight (at 0.5 m from the start, Table 2).

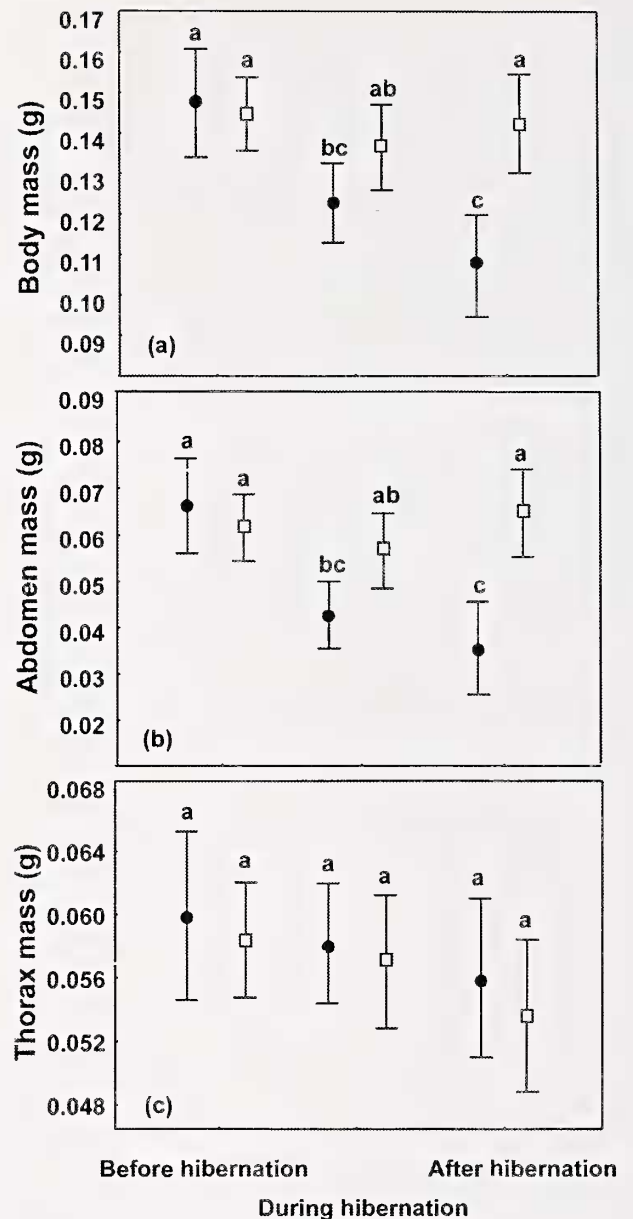
Female take-off angles were significantly lower both during and after hibernation compared to before hibernation. In contrast, velocity did not differ between seasons in females (Table 3, Fig. 2). There was, however, a significant effect of relative thorax mass on female velocity (Table 3) with linear regression establishing a positive relationship between residual thorax mass and velocity (at 0.5 m:  $r=0.46$ ,  $p=0.003$ ,  $r^2=0.23$ ).

Male butterflies tested before hibernation flew slower than males tested during hibernation (Table 2, Fig. 2), and showed a trend towards being slower than males after hibernation (Tukey HSD,  $p=0.09$ ). Male flight speed significantly increased during hibernation, however, take-off angles during hibernation were significantly reduced compared to before and after hibernation (Table 3, Fig. 2). Although body mass changed with season, relative flight muscle mass in male butterflies did not explain differences in velocity and take-off angle (Table 3).

#### DISCUSSION

Our results showed that the take-off performance of an adult hibernating butterfly is influenced by season and sex. Male butterflies increased flight speed during hibernation compared to before hibernation, but showed a reduction in take-off angles during hibernation. In contrast, female butterflies maintained flight speed across seasons but showed decreased take-off angles during and after hibernation. Female flight speed was positively correlated to relative flight muscle investment whereas the male flight pattern was mainly explained by season.

The body composition of male butterflies and the flight ability of both male and female butterflies in



**Figure 1. A-C.** Mean body mass (A), abdomen mass (B) and thorax mass (C) for male (filled circles) and female butterflies (open squares) across seasons. Different letters indicate significant differences at the 0.05 level established with Tukey HSD.

this study changed across seasons. Male butterflies lost weight during and after hibernation as predicted, with male body mass during hibernation being on average 19.2% less than that of males tested before hibernation, a figure well in line with the estimates of pre-hibernation lipid accumulation made by Pullin (1987). However, despite the expected reduction in male body mass both during and after hibernation, flight speed and take-off angles in males did not

**Table 1.** Summary statistics from ANOVA with body measurement as dependent variable and sex and season (before, during and after hibernation) as categorical factors. Differences between variables established with Tukey HSD are illustrated in Fig. 1 A-C. P<0.05 highlighted in bold.

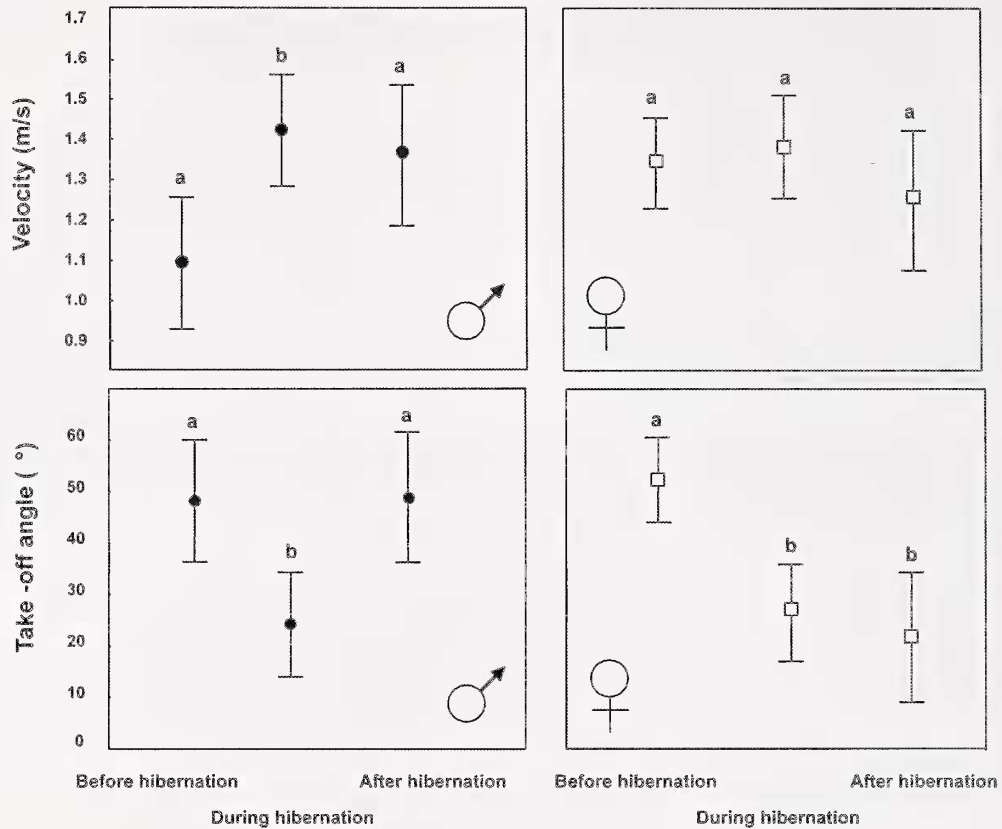
Factor	Df	Body mass		Abdomen mass		Thorax mass	
		F	p	F	p	F	p
Sex	1	10.9	<b>0.002</b>	12.9	<b>0.001</b>	0.9	0.3
Season	2	7.5	<b>0.001</b>	7.0	<b>0.002</b>	1.7	0.2
Sex x Season	2	5.0	<b>0.009</b>	6.8	<b>0.002</b>	0.03	0.9

**Table 2.** Effect of season, sex and flight muscle investment on flight ability in all attacked butterflies. Summary statistics of repeated measures GLM with season (before, during and after hibernation) and sex as categorical predictors, and thorax mass and body mass as continuous predictors. DS=distance from start. Differences between variables established with Tukey HSD are illustrated in Fig. 2. P<0.05 highlighted in bold.

Factor	Df	Take-off angle		Velocity	
		F	p	F	p
Body mass	1	3.2	0.078	0.3	0.558
Thorax mass	1	1.0	0.321	3.2	0.077
Sex	1	3.6	0.062	1.1	0.306
Season	2	9.0	<b>&lt; 0.001</b>	3.0	0.059
Sex x Season	2	5.7	<b>0.006</b>	2.3	0.110
DS	4	12.5	<b>&lt; 0.001</b>	1.0	0.387
DS x Body mass	4	4.9	<b>0.001</b>	1.6	0.186
DS x Thorax mass	4	0.4	0.835	2.4	0.051
DS x Sex	4	0.8	0.513	3.0	<b>0.021</b>
DS x Season	8	3.1	<b>0.002</b>	1.8	0.080
DS x Sex x Season	8	1.2	0.286	0.7	0.660

**Table 3.** Effect of season and flight muscle investment on male and female flight ability respectively. Summary statistics of repeated measures GLM with season (before, during and after hibernation) as categorical predictor, and thorax mass and body mass as continuous predictors. DS=distance from start. Differences between variables established with Tukey HSD are illustrated for each sex respectively in Fig. 2. P<0.05 highlighted in bold.

Factor	Df	Males				Females			
		Take-off angle		Velocity		Take-off angle		Velocity	
		F	p	F	p	F	p	F	p
Body mass	1	0.1	0.829	0.5	0.472	4.1	0.051	1.4	0.242
Thorax	1	1.6	0.220	0.2	0.703	0.4	0.517	7.3	<b>0.011</b>
Season	2	8.5	<b>0.002</b>	3.6	<b>0.046</b>	9.1	<b>0.001</b>	0.1	0.868
DS	4	2.6	<b>0.041</b>	1.3	0.276	10.3	<b>&lt;0.001</b>	0.8	0.544
DS x Body mass	4	0.3	0.0883	1.3	0.279	5.0	<b>0.001</b>	1.4	0.247
DS x Thorax mass	4	0.1	0.984	1.7	0.157	0.4	0.807	2.1	0.089
DS x Season	8	0.7	0.736	2.0	0.054	1.8	0.078	0.9	0.464



**Figure 2.** Mean velocity of male (filled circles) and female (open squares) butterflies across seasons (top). Mean take-off angles of male and female butterflies across seasons (bottom). Bars indicate 0.95 C.I. and different letters indicate significant differences at the 0.05 level established with Tukey HSD.

directly follow this pattern. Although flight speed and angles were both high after hibernation as expected, the patterns found when we compared flights before and during hibernation were opposing. Whereas escape flights before hibernation were characterised by steeper take-off angles and lower speed, the flights performed during hibernation showed an increase in speed and a reduction in angles. Thus, despite a reduction in load carried which would have increased relative flight muscle mass, male butterflies appeared to maintain flight speed at the expense of take-off angles during hibernation. One explanation for the reduction in take-off angles seen despite weight loss could be that the inactivity of flight muscles during hibernation may have temporarily reduced flight capacity in male butterflies; an explanation supported by work showing that such degeneration in hibernating insects and bats is reversible (Stegwee *et al.*, 1963; Kim *et al.*, 2000) and the fact that male escape flight after hibernation was both fast and steep, and not explained by a relatively larger flight muscle mass.

In contrast to male butterflies, females did not

exhibit any significant change in mass across seasons. Our expectation was that females would experience some reduction in body mass due to lipid depletion at least during hibernation; as females tested after hibernation were most likely mated no body mass reduction was expected. While a lack of body mass loss during hibernation may seem surprising, it must be noted that the butterflies tested before hibernation were sampled in a different year than the butterflies tested during and after hibernation. The years sampled may thus have provided different climate conditions that could have affected for instance lipid accumulation, hibernation length and severity, and general activity levels that could explain why we observed no mass loss in females. Regardless, despite a lack of weight loss, the flight pattern of female butterflies shifted across seasons, showing a significant decrease in take-off angles during and after hibernation. Because there was no observed reduction in flight speed, females, as well as males during hibernation, may have been adjusting take-off angles to promote faster flight. The benefit of a

reduction in take-off angles is conservation of energy as a steeper take-off is more energetically expensive and a lowering of angles also enhances acceleration capacity (Dudley, 2000). As previously mentioned, the reduction in flight angles during hibernation may also have been caused by temporary degeneration of flight muscles. Although abdomen and thorax mass of females did not change across seasons, it cannot be ruled out that the transfer of reproductively related loads between the sexes caused a shift in the centre of mass that may have affected their flight performance (Srygley & Dudley, 1993).

We expected that unless thorax mass had decreased during or after hibernation, flight ability would increase or be unaltered as body mass was reduced or maintained. Neither males nor females in our study differed in thorax mass across seasons, suggesting that resources from the thorax are not used to a large extent as a source of energy or reallocated to reproduction which occurs in some butterfly species (Stjernholm & Karlsson, 2006). The fact that female, but not male, flight speed was positively related to relative flight muscle mass suggests that female butterflies indeed are more sensitive to the trade-off between flight muscle investment and allocation of resources to reproduction than are males (Roff, 1984). Because of the continuous large load carried by females, the energetic cost for flight is probably also much higher than for males (Dudley, 2000). However, such energetic costs imposed on females may be circumvented in the long term as butterflies with relatively low flight muscle mass have been shown to reduce flight activity (Kingsolver & Srygley, 2000), a strategy that perhaps also serves to minimize the risk of encountering predators. Despite their lesser flight muscle investment, female flight during hibernation was equal to that of males, suggesting that females work harder (i.e. increased wing beat frequency) to achieve similar speed and angles. However, a study by Berwaerts and colleagues (2006) on tethered flight in *Pararge aegeria* found no difference in wing beat frequency between the sexes. Butterfly flight is often found to be sex-specific (Berwaerts *et al.*, 2006) but may depend on the type of flight under study. In this study, we focused on take-off flight due to its fitness relevance for predator evasion (Chai & Srygley, 1990) and mate acquisition (Van Dyck, 2003). It is, however, possible that the benefit of investment in relative flight muscle mass in males is revealed under flight types other than take-off; for instance, Nymphalid males such as *A. urticae* often engage in lengthy courtship flights that greatly demand agility and endurance. Furthermore, although their flight ability may exceed that of females during reproduction, males

in search for mates spend more time in flight than females (Shreeve, 1984), and may be at higher risk of predation, especially since extensive flight results in suboptimal body temperatures, which impairs take-off ability (Berwaerts & Van Dyck, 2004). It is worth noting that the temperature in the experimental arena and the relatively short time allowed for warm up most likely resulted in suboptimal body temperatures in the butterflies in our study, which may have more clearly revealed differences between the sexes (Berwaerts *et al.*, 2008).

Because the butterflies used in this study were obtained from the field, no reliable data on age could be collected. However, due to the duration of hibernation in this species, there was nonetheless a substantial difference in age across seasons (mainly before and after hibernation, as individuals tested during and after hibernation were of similar age), which may have influenced take-off flight performance. Increased adult age in butterflies has been associated both with lowered flight endurance (Åhman & Karlsson, 2009) and enhanced competitive success in flight contests (Kemp *et al.*, 2006). Flight ability has also been predicted to improve with age as body mass decreases and relative flight muscle mass increases over time (Stjernholm *et al.*, 2005). Males in this study increased relative flight muscle mass and showed no reduction in take-off performance after hibernation; thus, while take-off ability did not appear to be negatively affected by age, it was not explained by relative flight muscle mass in males. The lowered take-off performance of females during and after hibernation, despite the absence of any body mass change, could on the other hand be related to increased age, perhaps due to physiological changes of the functionality of the flight muscles (Saito, 2000) or depleted energy supplies.

Finally, our study confirms previous results showing that butterflies adjust flight effort depending on the perceived predation risk (Almbro & Kullberg, 2008). Kullberg and Lafrenz (2007) found that great tits attacked by a model predator reduced take-off angles in the presence of protective cover which allowed them to perform faster escape flights; in the absence of cover, take-offs were steeper which is suggested to allow a small prey to out climb a large predator (Hedenström & Rosén, 2001). Because the laws of gravity are the same for all flying animals, butterflies may very well differ in their flight response depending on the presence and absence of cover and type of predator. In our study, all flights were carried out without cover, and the significant net upward movement suggest that butterflies aim to out climb their attacker, possibly making the reductions in

take-off angles during hibernation a liability during a predator encounter.

In summary, our results show that butterfly take-off ability was primarily affected by season, sex and perceived predation risk. The importance of investing in a relatively large flight apparatus was evident in female, but not in male butterflies. Female flight ability after hibernation was characterised by maintained speed but lowered take-off angles whereas males shifted from low speed and steep angles before hibernation to low angles and higher speed during, with a surge in angles again after hibernation. Finally, our study confirmed that butterflies attacked by a model predator flew at greater speeds than during routine take-off.

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## LITERATURE CITED

- ÅHMAN, M. & B. KARLSSON. 2009. Flight endurance in relation to adult age in the green-veined white butterfly *Pieris napi*. *Ecological Entomology* 34: 783-787.
- ALMBRO, M. & C. KULLBERG. 2008. Impaired escape flight ability in butterflies due to low flight muscle ratio prior to hibernation. *Journal of Experimental Biology* 211: 24-28.
- ALMBRO, M. & C. KULLBERG. 2009. The downfall of mating: the effect of mate-carrying and flight muscle ratio on the escape ability of a pierid butterfly. *Behavioural Ecology and Sociobiology* 63: 413-420.
- BERGLUND, A. & G. ROSENQVIST. 1986. Reproductive costs in the prawn *Palaemon adspersus*: effects on growth and predator vulnerability. *Oikos* 46: 349-354.
- BERWAERTS, K. & H. VAN DYCK. 2004. Take-off performance under optimal and suboptimal thermal conditions in the butterfly *Pararge aegeria*. *Oecologia* 141: 536-545.
- BERWAERTS, K., P. AERTS & H. VAN DYCK. 2006. On the sex-specific mechanisms of butterfly flight: flight performance relative to flight morphology, wing kinematics, and sex in *Pararge aegeria*. *Biological Journal of the Linnean Society* 89: 675-687.
- BERWAERTS, K., E. MATTHYSEN & H. VAN DYCK. 2008. Take-off flight performance in the butterfly *Pararge aegeria* relative to sex and morphology: a quantitative genetic assessment. *Evolution* 62: 2525-2533.
- CHAI, P. & R. B. SRYGLEY. 1990. Predation and the flight, morphology, and temperature of neotropical rain-forest butterflies. *American Naturalist* 135: 398-411.
- DUDLEY, R. 2000. *The biomechanics of insect flight: form, function, evolution*. Princeton University Press, Princeton.
- EVANS, J. P., C. GASPARINI & A. PILASTRO. 2007. Female guppies shorten brood retention in response to predator cues. *Behavioral Ecology and Sociobiology* 61: 719-727.
- GHALAMBOR, C. K., D. N. REZNICK & J. A. WALKER. 2004. Constraints on adaptive evolution: the functional trade-off between reproduction and fast-start swimming performance in the Trinidadian guppy (*Poecilia reticulata*). *American Naturalist* 164: 38-50.
- HEDENSTRÖM, A. & M. ROSÉN. 2001. Predator versus prey: on aerial hunting and escape strategies in birds. *Behavioural Ecology* 12: 150-156.
- KEMP, D. J., C. WIKLUND & H. VAN DYCK. 2006. Contest behaviour in the speckled wood butterfly (*Pararge aegeria*): seasonal phenotypic plasticity and the functional significance of flight performance. *Behavioural Ecology and Sociobiology* 59: 403-411.
- KIM, M. H., K. PARK, B. J. GWAG, N. JUNG, Y. K. OH, H. SHIN & I. CHOI. 2000. Seasonal biochemical plasticity of a flight muscle in a bat, *Murina leucogaster*. *Comparative Biochemistry and Physiology Part A* 126: 245-250.
- KINGSOLVER, J. G. & R. B. SRYGLEY. 2000. Experimental analyses of body size, flight and survival in pierid butterflies. *Evolutionary Ecology Research* 2: 593-612.
- LAYNE JR., J. R. & M. E. RICE. 2003. Post-freeze locomotion performance in wood frogs (*Rana sylvatica*) and spring peepers (*Pseudacris crucifer*). *Canadian Journal of Zoology* 81: 2061-2065.
- MORSE, D. H. 1975. Ecological aspects of adaptive radiation in birds. *Biological Reviews* 50: 167-214.
- PLAUT, I. 2002. Does pregnancy affect swimming performance of female mosquitofish, *Gambusia affinis*? *Functional Ecology* 16: 290-295.
- PULLIN, A. S. 1987. Adult feeding time, lipid accumulation, and overwintering in *Aglaia urticae* and *Inachis io* (Lepidoptera: Nymphalidae). *Journal of Zoology* 211: 631-641.
- ROFF, D. A. 1984. The cost of being able to fly: a study of wing polymorphism in two species of crickets. *Oecologia* 63: 30-37.
- SAITO, O. 2000. Flight activity changes of the cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), by aging and copulation as measured by flight actograph. *Applied Entomology and Zoology* 35: 53-61.
- SHAFFER, L. R. & D. R. FORMANOWICZ JR. 1996. A cost of viviparity and parental care in scorpions: reduced sprint speed and behavioural compensation. *Animal Behaviour* 51: 1017-1024.
- SHINE, R. 1980. Costs of reproduction in reptiles. *Oecologia* 46: 92-100.
- SHREEVE, T. G. 1984. Habitat selection, mate location and microclimatic constraints on the activity of the speckled wood butterfly *Pararge aegeria*. *Oikos* 43: 371-377.
- SRYGLEY, R. B. & R. DUDLEY. 1993. Correlations of the position of centre of body mass with butterfly escape tactics. *Journal of Experimental Biology* 174: 155-166.
- SRYGLEY, R. B. & J. G. KINGSOLVER. 1998. Red-wing blackbird reproductive behaviour and the palatability, flight performance and morphology of temperate pierid butterflies (*Colias*, *Pieris*, and *Pontia*). *Biological Journal of the Linnean Society* 64: 41-55.
- STEGWEE, D., E. C. KIMMEL, J. A. DE BOER & S. HENSTRA. 1963. Hormonal control of reversible degeneration of flight muscle in the Colorado potato beetle, *Leptinotarsa decemlineata* Say (Coleoptera). *Journal of Cell Biology* 19: 519-527.
- STJERNHOLM, F. & B. KARLSSON. 2006. Reproductive expenditure affects utilization of thoracic and abdominal resources in male *Pieris napi* butterflies. *Functional Ecology* 20: 442-448.
- STJERNHOLM, F., B. KARLSSON & C. L. BOGGS. 2005. Age-related changes in thoracic mass: possible reallocation of resources to reproduction in butterflies. *Biological Journal of the Linnean Society* 86: 363-380.
- SVÄRD, L. & C. WIKLUND. 1989. Mass and production rate of ejaculates in relation to monandry/polyandry in butterflies. *Behavioural Ecology and Sociobiology* 24: 395-402.
- SWADDLE, J. P. & M. S. WITTER. 1997. The effect of moult on the flight performance, body mass, and behaviour of European starlings (*Sturnus vulgaris*): an experimental approach. *Canadian Journal of Zoology* 75: 1135-1146.
- VAN DYCK, H. 2003. Mate-location: a matter of design? Adaptive morphological variation in the speckled wood butterfly. *In: Butterflies: Ecology and evolution taking flight*. University of Chicago Press, Chicago, USA.
- WICKMAN, P.-O. 1992. Sexual selection and butterfly design - a comparative study. *Evolution* 46: 1525-1536.